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CONCERNING THE QUENCHING OF THE FLUORESCENCE OF CHLOROPHYLL AND MAGNESIUM PHTHALOCYANIN AND THE INTERACTION OF THESE PIGMENTS WITH THE QUENCHING SUBSTANCE

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An investigation of the quenching of fluorescence of dyestuffs [pigments] by other molecules will clarify the nature of the photochemical effect exerted by dyestuffs in general and chlorophyll in particular. In a prior communication [1] we published data on the quenching of the fluorescence of magnesium phthalocyanin in alcoholic solution. It follows from the results published there that the molecules of oxidizing agents exhibit the strongest quenching action. Livingston and Chun-Lin Ke [2] arrived at the same conclusion after studying the effect of a great number of substances on chlorophyll in methanol solution.

In the present communication the effects of various substances on the intensity of fluorescence of chlorophyl (a + b) and Mg phthalocyanin in solution were studied using the following solvents: toluene, methanol, and pyridine. The interaction of the quenching substance with the molecules of the pigment was also investigated. The solution of Mg phthalocyanin in toluene was prepared by using ether first, as described earlier [6].

Fluorescence was induced in the interval of maximum absorption for the substances in question in the red region of the spectrum and the intensity of the fluorescence was measured by a method described earlier [1]. The solutions were filled into vacuum test tubes which had a special shape and were adapted to direct measurement of absorption by means of a spectrophotometer. The dissolved oxygen was removed by means of an oil pump while the solvent was boiled.

Table 1 cites data showing the variations in the intensity of fluorescence and the extent of absorption at the red maximum exhibited by solutions of the pigments in question under the action of various substances. The concentration of the latter was in all cases 0.1 M per liter.

The absorption values E shown in Table 1 were measured directly in the test tubes by means of a photelectric spectrophotometer. The results listed listed in table confirm for other solvents besides alcohol that oxidants have the strongest quenching effect. Reducing agents generally do not diminish the intensity of fluorescence. When they do, their effect is much weaker than that of oxidants.

The interaction between the molecules of the quenching substance and the pigment can be evaluated on the basis of the shift and change of relative value of the maxima on the spectral absorption curve. Strongly quenching oxidants like quinine and oxygen do not change the appearance of the absorption spectrum of pigments in the usual solvents, while in the case of iodine or dinitrobenzene the magnitude of absorption at the red maximum diminishes noticeably, indicating a chemical interaction. This leads to the conclusion that quenching by oxidants involves several different mechanisms.

In order to investigate the connection between the quenching of fluorescence and ability of the molecules of chlorophyll and those of the quenching substance to interact photochemically in the case when they apparently do not react with each other in the dark (at which one could conclude from the fact that the absorption spectrum did not change), the following experiments were set up.

Solutions of chlorophyll which contained quinone, hydroquinone, or ascorbic acid were freed from oxygen by removing the latter with an oil pump while the solution was boiled. The solutions were then illuminated during 9 minutes by a 500 watt motion picture lamp which was placed at a distance of 45 centimeters. The light was focused by means of a condenser and was passed through an RG-2 red filter 10 mm thick.

The results of these experiments, as well as data on the photochemical oxidation by oxygen of the air under the same conditions, are shown in the curves of Figure 1. The ordinates of these curves represent absorption values at the red maximum in percent of absorption by the solution which has not been exposed to the action of light. The results in question indicate that there is no direct connection between the quenching of fluorescent and the ability of chlorophyll to interact chemically with the quenching substance. For instance, quinone quenches fluorescence energetically, although it practically does not react at all photochemically with chlorophyll either in alcohol or toluene. On the other hand, ascorbic acid does not affect the intensity of chlorophyll fluorescence, while it reacts much more rapadily than quinone in alcohol and very rapidly in pyridine.

Although the photoreaction of chlorophyll with ascorbic acid in pyridine is a reversible photoreduction [4], the relative slowness of the reverse reaction under anaerobic conditions permits one to trace the course of the photoreaction by measuring E after the light has been switched off.

In order to check whether a very rapid reversible chemical reaction does not take place between 1) a strongly quenching substance which does not change the appearance of the chlorophyll absorption spectrum and 2) the dyestuff, we set up an experiment in which changes in the absorption of 0.1 M/l chlorophyll (a + b) solutions in alcohol, pyridine, or toluene containing 0.1 M of quinone

per liter were observed under illumination of these solutions by strong light directed perpendicularly to the ray of light passing through the spectrophotometer's monochromator. The assembly used in this experiment is shown in Figure 2.

For the purpose of avoiding errors due to the scattering of light and fluorescence, the lateral illumination of the solution was transmitted through an RG-2 red filter transmitting wave lengths higher than 620 mμ, while observation of absorption changes was conducted in the region of the second chlorophyll maximum at 430-440 mμ. The experiment demonstrated that switching on of the lateral illumination never changed absorption at the blue chlorophyll maximum, thus proving the absence of a chemical reaction.

As a result of this work, the following conclusions were reached:

1) Oxidants exert a stronger quenching effect than other substances on the fluorescence of chlorophyll in alcohol, pyridine, and toluene solutions;

2) There is no direct connection between the quenching effect of extraneous molecules and their ability to react photochemically with chlorophyll, which indicates that the ability to react is connected with a comparatively durable biradical (triplet) state rather than the electronically excited state that has a life of only 10^{-7} - 10^{-8} seconds [5].

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Table 1. Changes in the Intensity of Fluorescence and of the Value of Absorption at the Red Maximum of Chlorophyll (a + b) and Phthalocyanin Solutions as a Result of Adding Other Substances.

Substance added	Solvent	Chlorophyll (a + b)					Magnesium phthalocyanin				
		I_1	I_2	% of extinction	E_1*	E_2	I_1	I_2	% of extinction	E_1*	E_2
Nitrobenzene	Ethanol	42.5	13.6	68	0.484	0.479	66.5	20	70	0.495	0.486
m-Dinitrobenzene	"	42.5	5.5	87	0.458	0.393	65.5	9.0	86	0.492	0.469
I_2 (0.005 M)	"	42.7	19.5	53	0.650	0.550	71.4	29.8	58	-	-
Ascorbic acid	"	46.1	46.1	0	0.409	0.409	53.0	54.0	0	0.390	0.392
Benzoic acid	"	45.0	45.0	0	0.486	0.483	69.0	69.0	0	0.494	0.509
Aniline	"	44.5	45.0	0	0.480	0.473	65.1	50.0	23	0.498	0.491
Dimethylaniline	"	35.4	25.5	28	0.780	0.750	62.5	18.4	71	0.477	0.462
Phenol	"	45.0	45.0	0	-	-	68.0	68.0	0	0.498	0.491
Benzaldehyde	"	42.0	42.0	0	0.480	0.455	67.0	67.0	0	0.495	0.481
Catechol	"	46.0	47.0	0	0.470	0.470	66.5	66.5	0	0.499	0.481
Pyrogallol	"	46.0	46.0	0	0.480	0.478	68.5	66.5	3	0.489	0.485
Phenylhydrazine hydrochloride (0.5M)	"	47.0	43.0	8	0.462	0.456	66.0	66.0	0	0.528	0.510
p-Quinone	Pyridine	49.2	2.7	94.5	0.660	0.660	44.6	1.7	96	0.648	0.648
Hydroquinone	"	49.1	50.2	0	0.650	0.648	44.1	41.6	6	0.655	0.646
Nitrobenzene	"	48.0	30.2	37	0.640	0.614	43.8	30.2	31	0.675	0.661
m-Dinitrobenzene	"	47.4	10.4	78	0.620	0.594	45.3	6.5	86	0.671	0.645
Aniline	"	45.0	44.9	0	0.620	0.605	44.0	42.1	4	0.656	0.646
Dimethylaniline	"	45.2	44.4	2	0.612	0.593	43.3	27.3	37	0.661	0.627
Ascorbic acid	"	44.0	44.0	0	0.480	0.480	-	-	-	-	-
p-Quinone	Toluene	49.1	3.3	93	0.680	0.683	48.0	2.0	96	0.570	0.560
Nitrobenzene	"	47.1	33.5	29	0.675	0.663	48.0	24.0	50	0.590	0.581
m-Dinitrobenzene	"	49.2	11.7	75	0.655	0.611	48.0	10.0	81	0.592	0.562
Aniline	"	47.5	48.0	0	0.648	0.641	48.0	49.0	0	0.583	0.576
Dimethylaniline	"	46.1	47.5	0	0.647	0.630	47	39	17	0.586	0.568

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Glossary for Table 1.

I_1 = relative magnitude of the intensity of fluorescence; E_1 = absorption at the maximum in the case of a solution to which no extraneous substance had been added; I_2 and E_2 = the same values for solutions to which another substance had been added.

* Position of the red maximum in ethanol: chlorophyll 665 mμ, Mg phthalocyanin 668 mμ. The same in pyridine: chlorophyll 670 mμ, Mg phthalocyanin 673 mμ. The same in toluene: chlorophyll 665 mμ, Mg phthalocyanin 672 mμ.

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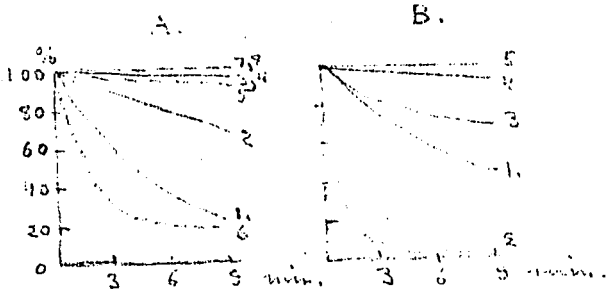

Figure 1. Photochemical Reactions of Chlorophyll.

A. In ethanol: 1 - O_2; 2 - ascorbic acid; 3 - quinone; 4 - hydroquinone; 7 - solution to which no second substance had been added. In toluene; 5 - quinone; 6 - O_2; 8 - solution to which no second substance had been added.

B. In pyridine: 1 - O_2; 2 - ascorbic acid; 3 - quinone; 4 - hydroquinone; 5 - solution to which no second substance had been added.

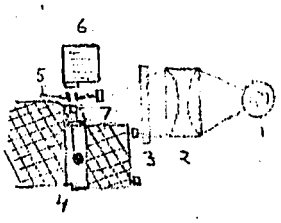

Figure 2. Arrangement of the Apparatus in Experiments with Lateral Illumination. 1 - 500 watt motion picture lamp; 2 - condenser; 3 - RG-2 filter; 4 - spectrophotometer; 5 - optical slit; 6 - source of light for the spectrophotometer; 7 - holder for the test tube containing solution.

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